Letters to the Editor

Contamination of dry powder inhalers for asthma with milk proteins containing lactose

To the Editor:

Milk allergy is one of the most common food allergies, affecting approximately 2.5% of infants and young children. Most children, approximately 80%, outgrow milk allergy by age 5 years; however, some patients have persistent allergy.[1] Lactose is obtained from cow's milk and is commonly used as an inactive ingredient in many pharmaceutical formulations including tablets, suspensions, and dry powder inhalers for asthma (DPIs). Patients with severe milk allergy frequently ask whether pharmaceutical products containing lactose are safe for them to use and whether there is a possibility of milk contamination.

We became aware of anecdotal evidence that some patients with severe milk allergy had allergic reactions after the ingestion of lactose-containing medications. These reactions were attributed to milk protein contamination in the lactose filler. In this report, we describe a patient who had repeated and objectively documented anaphylactic reactions after inhalation from a new lot of Advair Diskus despite having tolerated Advair for months previously. We also report the results of immunologic studies that demonstrate the presence of milk proteins in DPI-containing lactose and in pharmaceutical grade lactose (lactose USP).

An 8-year-old boy with severe milk allergy and persistent asthma was maintained on Serevent (salmeterol) metered-dose inhaler (MDI) and Flovent (fluticasone) MDI. However, when combined therapy became available, he was successfully transitioned to Advair Diskus, a single DPI containing both salmeterol and fluticasone. He had multiple allergic reactions from ingestion or contact with minute amounts of milk proteins in the past. The patient continued to receive Advair for several months without any adverse reactions and with excellent asthma control. However, after inhalation of three consecutive doses from a new diskus, he immediately complained of chest tightness and feeling of distress that were treated with oral diphenhydramine and inhaled bronchodilator at home. The patient subsequently underwent a supervised inhalation challenge in his allergist's office, where he had immediate chest tightness, decline in FEV_1 from 1.67 to 0.58 L/min, and blood pressure drop from 90/58 to 64/40 mm Hg after the inhalation of one dose from the incriminated Advair Diskus. He recovered with intramuscular epinephrine, diphenhydramine, and prednisone. The patient was switched back to Serevent MDI and Flovent MDI without further complications.

Subsequently, powder was obtained from different lots of Advair. Proteins were extracted with PBS and used for skin testing. Skin prick testing was done with the use of a sterile smallpox needle (Hollister Stier Laboratories, LLC) with the following results: commercial milk extract 7/40 (Hollister Stier Laboratories, LLC), lactose USP 5/20 (obtained from commercial pharmacy), Advair lot No. 1 3/26, histamine 5/22, Advair lot No. 2 0/8, glycerinated saline 0/0 (mean wheal diameter/mean erythema diameter, mm). Serum-specific milk- and casein-IgE antibody concentrations were measured with the Pharmacia CAP System, Pharmacia Diagnostics; the lower limit of detection is 0.35 kU/L and the upper limit of detection is 100 kU/L. The patient's serum-specific milk-IgE was 17.9 kU/L (milk-IgE antibody concentration >15 kU/L has 95% positive predictive value for an acute allergic reaction to milk upon ingestion); casein-IgE was 24.3 kU/L.

Subsequently, Advair and other lactose-containing DPIs were tested for the presence of milk proteins. Protein fractions from DPIs were extracted with PBS, purified, and concentrated with the use of precipitation technique Page-Perfect, Genotechnology, Inc, St Louis, Mo. A sensitive inhibition-ELISA assay was performed as previously described and detected milk proteins in the tested DPIs; however, precise quantification of milk protein content was not possible because of the apparent interference, presumably from large quantities of lactose causing glycosylation of milk proteins with subsequent changes in protein conformation. The presence of proteins that were of similar molecular weight as milk β-lactoglobulin in the tested DPIs containing lactose was detected with silver staining (Fig 1, *A*). Subsequently, specific labeling with monoclonal antibodies against milk proteins (generously provided by Dr Patrizia Restani from Institute of Pharmacological Sciences in Milan, Italy) as well as immunolabeling with sera from patients with severe milk allergy detected caseins in Advair and β-lactoglobulin in Foradil. Both milk proteins were also present in the lactose USP (Fig 1, *B* and *C*).

In this report, we described an asthmatic patient with documented allergy to cow's milk, who had anaphylactic reactions to milk protein contaminating Advair Diskus. Pharmaceutical grade lactose is obtained from skim milk by coagulating and filtering out milk protein. The efficiency of this process is apparently quite high, and the product information inserts do not caution patients with milk allergy about the possibility of an allergic reaction to milk proteins in lactose-containing medications. Likewise, milk allergy is not included among the contraindications against using lactose-containing pharmaceutical formulations.

Dry powder inhalers are becoming mainstream asthma therapy. Both rescue and maintenance anti-inflammatory medications are available in the DPI formulation. It is expected that within several years DPIs will capture a large share of the inhaled asthma medications market as a result of the Montreal Protocol and chlorofluorocarbon phase-out. Currently, except for Pulmicort Turbuhaler, all other DPIs available in the United States contain from 12.5 mg (Serevent Diskus, Advair Diskus) to 25 mg (Foradil Aerolizer, Flovent Rotadisk, Ventolin Rotacaps) pharmaceutical grade lactose per dose.

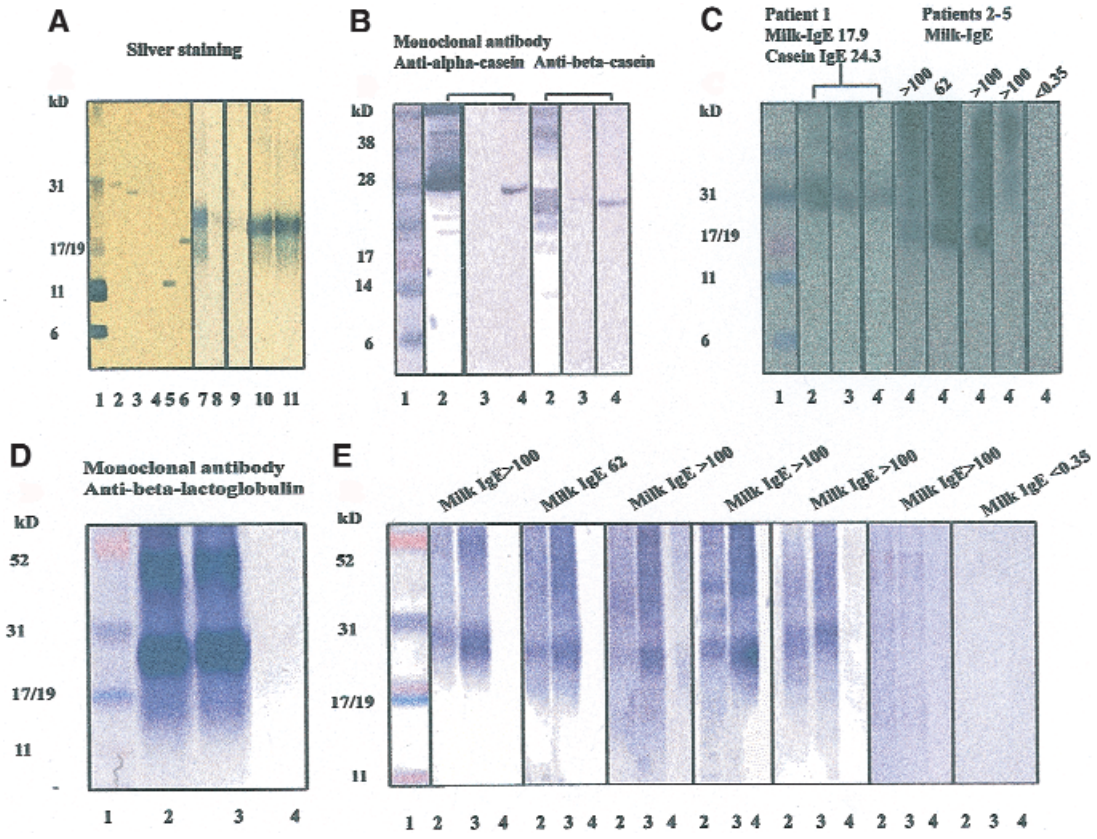

FIG 1. A, Silver staining of protein content in selected DPIs compared with silver staining of cow's milk proteins. Proteins of molecular weight similar to β-lactoglobulin are present in Lactose USP, Foradil, and Flovent: *1,* molecular weight standard; *2,* α-casein; *3,* β-casein; *4,* κ-casein; *5,* α-lactalbumin; *6,* β-lactoglobulin; *7,* lactose USP; *8,* Advair (12.5 mg lactose/dose); *9,* Serevent (12.5 mg lactose/dose); *10,* Flovent (25 mg lactose/dose); *11,* Foradil (25 mg lactose/dose). **B,** Casein detection in Advair and Lactose USP by labeling with monoclonal antibody against α-casein and β-casein; *1,* molecular weight standard; *2,* milk; *3,* lactose USP; *4,* Advair No. 1 (12.5 mg lactose/dose). **C,** Immunolabeling with sera of patients allergic to milk. *Patient 1* is the patient described in the case report; *patients 2 through 4* have severe milk allergy and high serum milk-IgE antibody concentrations, and *patient 5* is not allergic to milk and serves as a negative control; *1,* molecular weight standard; *2,* milk; *3,* lactose USP; *4,* Advair No. 1 (12.5 mg lactose/dose). **D,** β-Lactoglobulin detection in Foradil (25 mg lactose/dose) and lactose USP by labeling with monoclonal antibody against β-lactoglobulin; *1,* molecular weight standard; *2,* lactose USP; *3,* Foradil (25 mg lactose/dose); *4,* Pulmicort (no lactose). **E,** Immunolabeling with sera of patients allergic to milk. In addition to *patients 2 through 4* from **C,** two other subjects with severe milk allergy were included. Milk-tolerant patient with milk-IgE <0.35 kU/L serves as negative control; *1,* molecular weight standard; *2,* lactose USP; *3,* Foradil (25 mg lactose/dose); *4,* Pulmicort (no lactose).

The lactose excipient in DPIs improves the efficiency of the blister pack opening upon breath activation and improves the delivery of the drug into small airways. Lactose also indicates delivery of the drug as the result of its sweet taste. Lactose USP is obtained from skim milk according to strict industry standards. Casein is precipitated with diluted hydrochloric acid. After removal of the casein by filtration, the reaction of the whey is adjusted to a pH of approximately 6.2 by addition of lime, and the remaining albuminous matter is coagulated by heating (93.5°C); this is filtered out and the liquid is set aside to crystallize.[2]

The possible routes of milk protein exposure from DPIs include both inhalation and ingestion because >98% of lactose settles in the oropharynx and is swallowed. Although the threshold dose of inhaled milk protein has not been established, previous reports described children with severe milk allergy having acute allergic reactions after the ingestion of food products containing >10 parts per million of total milk protein.[3] It is well known that inhalation of cow's milk,[4] wheat,[5] and fish[6] can induce severe anaphylactic reactions in allergic individuals. Repeated exposure to low doses of inhaled milk proteins might also exacerbate chronic airway inflammation and lead to poor asthma control in the absence of acute immediate reactions.

Occupational asthma as a result of exposure to inhaled allergens has been reported in the bakery, confectionery, and pharmaceutical industries. Furthermore, a recent report documented development of egg-induced asthma and subsequent systemic symptoms caused by ingestion of egg in 3 previously egg-tolerant bakery and confectionery

workers after the exposure to airborne egg allergens in the workplace.[7] In addition, anaphylactic reactions to inhaled rice flour in a child who tolerated ingested rice were described.[8] Considering the high vascularity of the respiratory tract mucous membranes and lack of protective mechanisms unique to the gastrointestinal tract, such as digestive enzymes and low pH, one could hypothesize that the smaller doses of inhaled food allergen would induce an allergic reaction compared with an ingested allergen in highly sensitive individuals.

Another factor that may contribute to lower threshold for inhaled food allergens is that allergenicity of milk proteins may be enhanced by formation of the lactose-protein complexes. Nonenzymatic glycosylation of milk proteins occurs during heat treatment (Maillard reaction), leading to significant changes in the 3-dimensional structure of these proteins. These conformational modifications might lead to large glycoprotein complex formation and enhanced allergenicity. In fact, intradermal skin test reactivity to β-lactoglobulin–lactose conjugates has been shown to be 10- to 100-fold increased compared with native β-lactoglobulin.[9] Furthermore, large complexes may be randomly distributed explaining why some lots of Advair contained larger amounts of milk proteins compared with others. In addition, the purity of lactose USP may differ among the manufacturers as well as among the batches from the same source. A recent paper reported that none of the 24 children with well-characterized immediate cow's milk allergy reacted on a blinded challenge with soy-based infant formula containing lactose and that there was no detectable milk protein in a single batch of lactose provided by an Italian manufacturer.[10]

Based on the experience of our patient, who tolerated several lots of Advair before development of severe allergic reaction to an inhaler from a different lot, there appears to be clinically significant and apparently random variability between the lots of the DPIs in regard to milk protein contamination. It is therefore advisable that lactose-containing DPIs be used with caution in patients with severe milk allergy. Considering the unpredictable lot-to-lot variability in milk protein contamination, lactose-free DPI or alternative asthma inhalers should be preferred choices for such patients. Furthermore, product information inserts of the lactose-containing DPIs should include a warning on possible allergic reactions to milk protein contamination

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